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Quantitive Determination of Flavin Nucleotide Using the Bacterial Bioluminescent Reaction

The problem:

To develop a simple, reliable, and sensitive technique for assaying flavin coenzymes. By present methods, these enzymes, which are important in bio-medical research, have been difficult to assay with specificity and sensitivity. Accordingly, an improved technique for this purpose is highly desirable.

The solution:

A photometric method based on the use of bacterial luminescent reaction for detecting quantitatively the presence of flavin compounds in all forms of life.

How it's done:

Investigations of the feasibility of the bacterial bioluminescent reaction as a basis for an extraterrestrial life detection system have led to the development of a photometric technique for detecting quantities of flavin mononucleotide (FMN) as small as 0.00001 μg . Present methods that are used for assaying the flavin compounds, such as fluorometry and paper chromatography, lack the sensitivity and specificity that are desirable.

The instant technique involves a simple treatment of an aqueous cellular dispersion of a biological sample with an aqueous perchloric acid, which ruptures the cells and frees the flavin coenzymes from their proteins. In a total flavin-coenzyme assay, the flavin adenine dinucleotide (FAD) is hydrolyzed to FMN; however, the unhydrolyzed sample can be assayed for FMN alone. The FMN is then reduced with sodium borohydride and palladium chloride in solution, which is a required component of the *in vitro* bacterial bioluminescent reaction. The reduced compound (FMN-H₂) is then reacted with the enzyme luciferase to produce the luminescent effect. Under the con-

trolled conditions of the process, the intensity of the emitted light is in direct linear proportion to the concentration of FMN-H₂. The light intensity is measured by conventional methods that are readily available. Thus, a very sensitive and specific measure of the FMN concentration in the sample is determined.

Notes:

1. By this invention, the assay of flavin coenzymes is significantly simplified and provides exceptional specificity and sensitivity.
2. The process, which is now used in current work, can be adapted for use in both manned and unmanned space vehicles for detecting extraterrestrial life. It should also be of great value in metabolic research.
3. The following documentation may be obtained from:

Clearinghouse for Federal Scientific
and Technical Information
Springfield, Virginia 22151
Single document price \$3.00
(or microfiche \$0.65)

Reference: NASA Case No. GSC-10565-1
(N69-33349), Bioluminescent Assay Technique for Flavin Coenzymes

Patent status:

Inquiries about obtaining rights for the commercial use of this invention may be made to NASA, Code GP, Washington, D.C. 20546.

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